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Applying Imidacloprid Via a Precision Banding System to Control Striped Cucumber Beetle (Coleoptera: Chrysomelidae) in Cucurbits

J. JASINSKI,¹ M. DARR,² E. OZKAN,³ AND R. PRECHEUR⁴

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ABSTRACT The striped cucumber beetle, *Acalymma vittatum* (F.) (Coleoptera: Chrysomelidae), is a key pest of cucurbit crops throughout its range. A novel precision band applicator was designed to inject a solid stream of imidacloprid solution in-furrow directly over the seed during planting to reduce beetle leaf feeding on pumpkin, zucchini, and cucumber crops. In 2004 and 2005, bioassays at the cotyledon through fifth leaf were conducted on striped cucumber beetles using seedling leaf tissue grown from seeds treated using both continuous and precision banded in-furrow imidacloprid solution applications. In 2004, 80% of bioassay trials had treatments with beetle mortality significantly higher than the check, whereas 70% of the bioassay trials showed no significant difference in mortality between continuous in-furrow and precision banded treatments. In 2005, 79% of bioassay trials had treatments with beetle mortality significantly higher than the check, whereas 100% of the bioassays showed no significant difference in beetle mortality between continuous in-furrow and precision banded treatments at the same insecticide rate. The environmental savings of precision banded treatments compared with continuous in-furrow treatment reduced imidacloprid up to 84.5% on a per hectare basis for all cucurbits tested in 2004 and 2005, translating into an economic savings up to \$215/ha. In separate bioassay trials conducted in 2005 on pumpkin, where insecticide band length and injection volume were manipulated independently, several treatments had significantly higher beetle mortality than the check. There was a trend of increased beetle mortality in treatments using shorter band lengths combined with higher insecticide solution volumes.

KEY WORDS imidacloprid, cucurbit, bioassay, striped cucumber beetle

The striped cucumber beetle, *Acalymma vittatum* (F.) (Coleoptera: Chrysomelidae), is a key pest of cucurbit crops in midwestern and northeastern states. This insect can seriously injure or destroy emerging seedlings and is a known vector bacterial wilt, *Erwinia tracheiphila* (E. F. Smith), through fecal contamination of feeding wounds (Smith 1911, Leach 1964). Once the bacteria enter the plant, they multiply within the xylem, interfering with water translocation and ultimately leading to a wilted, dead plant. Susceptibility to bacterial wilt differs among cucurbits species with muskmelons, cucumbers, and 'Hubbard' and butter-nut squash more sensitive to infection compared with pumpkin, zucchini, and watermelon (Watterson et al. 1971, Brust and Palumbo 2005, Foster et al. 2005). Brust (1997) determined the susceptibility of pumpkins to bacterial wilt decreased with age, with no infections being detected after the second leaf stage.

Many pesticide and cultural control options exist for management of striped cucumber beetle in cucurbits. Pair (1997) used carbofuran-treated squash trap crops to intercept and kill beetles en route to canteloupe, squash, and watermelon fields in Oklahoma. In Maine, mixtures of squash trap crop and cucumber main crop found beetles concentrated on the trap crop (Radin and Drummond 1994). Experiments in Connecticut used an insecticide sprayed perimeter trap crop of 'Blue Hubbard' squash to protect cucumber plants against beetle feeding and had higher yields compared with check plots including mitigated bacterial wilt transmission to the interior of the field (J. Boucher, personal communication). The use of kairomone blends to attract, trap, and kill diabroticite and *Acalymma* spp. beetles have been researched in cucurbit hybrids, sweet potatoes, and pumpkins (Rhodes et al. 1980; Jackson et al. 2005; J.J. and C. Welty, unpublished). Delayed direct seeding or transplanting can also be used to avoid peak emergence of overwintering adult striped cucumber beetles.

Scouting protocols, action thresholds, and insecticide options have been established for managing striped cucumber beetle on a variety of cucurbit species based on pest density and developmental stage (Brust and Foster 1999, Brust and Palumbo 2005, Foster et al. 2005, Welty et al. 2007). The use of liquid or

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granular systemic insecticides applied directly in-furrow with the seed at planting is a preventative strategy currently available for use on all cucurbits. The systemic insecticide Furadan (carbofuran, FMC, Philadelphia, PA) has been shown to control striped cucumber beetles effectively and has been reported to act as a plant stimulant in watermelon (Buhler et al. 1992, Foster and Brust 1995). Wet field conditions can negatively impact carbofuran's performance by leaching it from the site of application thereby reducing the concentration below the minimal lethal dose to kill insect larvae in the root zone (Shelton et al. 1993). Environmentally, carbofuran has been noted as a low level contaminant in ground water in many states throughout the Corn Belt (Hallberg 1989) and is also susceptible to microbial degradation resulting in additional loss of efficacy (Felsot et al. 1981).

In 1992, a systemic neonicotinoid insecticide, imidacloprid (Bayer CropScience, Research Triangle Park, NC), was introduced to the U.S. market with wide spectrum control of aphids, whiteflies, termites, beetles, and other insects (Mullins 1993, Ware 2000). Currently labeled on the cucurbit crop group, this systemic insecticide can be applied as a drench to seedlings in the greenhouse immediately before field transplanting, chemigation through drip tape, or applied directly into the seed furrow at planting. Even at low concentrations, imidacloprid taken up into the seedling by the roots is distributed throughout the vegetative tissue. In potatoes, sugar beets, and sunflowers, the highest concentration of imidacloprid resides in the lower or older leaves and the lowest concentration is found among the upper and youngest leaves (Westwood et al. 1998, Laurent and Rathahao 2003, Olsen et al. 2004). In fact, the concentration difference between the bottom and upper leaves can be six- to-10-fold.

In cucurbits this compound has been shown to provide critical early season protection from striped cucumber beetle leaf feeding (Fleischer et al. 1998, Mac Intyre Allen et al. 2001). In addition to liquid application of imidacloprid, researchers in Ontario, Canada used Gaucho 480 F (imidacloprid, Bayer CropScience) as a seed treatment on cucumber and squash to reduce seedling damage from striped cucumber beetle (Mac Intyre Allen et al. 2001a).

Although a variety of cultural strategies and insecticides are available to manage striped cucumber beetles in cucurbits, few have concentrated on novel ways of compound delivery. As an alternative to seed treatment or continuous in-furrow application during planting, Hancock et al. (2004) developed a precision band applicator to inject a specific amount of fungicide over cotton seed in-furrow as it was being direct seeded. J.J. et al. (unpublished) at The Ohio State University (Columbus, OH) designed a similar precision band applicator to treat direct seeded pumpkin, zucchini, and cucumber seeds with insecticide as they enter the furrow. The precision band applicator functions by delivering a discrete 2–3-mm-diameter single solid stream of insecticide solution before, over the top of, and behind the seed in the open furrow before

being covered by the closer wheels. The stream of insecticide is not applied across the furrow in a fan as in traditional banding but as a single stream directed entirely inside the furrow.

In trials conducted in 2004 and 2005, the objectives were to determine the accuracy of the precision band applicator and calculate the environmental and economical savings of this application technology. A second objective was to use leaf bioassays to measure efficacy of both continuous in-furrow and precision banded technology using imadacloprid on treated pumpkin, zucchini, and cucumber seed by measuring percent striped cucumber beetle mortality. The last objective examines how band length and volume relative to the seed affect beetle mortality in leaf bioassays.

Materials and Methods

Precision Band Applicator. The precision band applicator was designed and assembled in the Department of Food, Agriculture, and Biological Engineering laboratory (The Ohio State University, Columbus, OH). The applicator was assembled from commercially available parts (spray hose, wiring harness, 12-Volt pump, tank, and so on) with the exception of a miniature solenoid valve (Capstan Ag Systems, Topeka, KS) placed immediately before the nozzle which controlled liquid flowing through the spray tip. The nozzle itself was composed of a Quick TeeJet Cap and orifice plate CP 4916–80 (Teejet, Springfield, IL), which produced a single solid stream of liquid. The row controller was custom designed by the lead agricultural engineer to activate the miniature solenoid. The precision band applicator was mounted to a Monosem single row vacuum planter (Lenexa, KS) for field trials.

The precision banding process is initiated once seed exits the seed metering box on the planter and begins falling through the seed delivery tube past a standard optical sensor toward the soil surface. In this research application, the sensor signal is directed to the injector controller, which initiates the insecticide application process by turning on and off the miniature solenoid valve mounted directly behind the spray nozzle head located between the depth control wheels. The injector delivers the insecticide solution for a specific period of time in a single solid stream at 82.7–103.4 kPa directed at the bottom of the seed furrow. The banding stream is turned on before the seed reaches the soil surface, then over the seed as it falls into the furrow, and continues past the seed for a predetermined time, then shuts off. The precision bander is calibrated to treat the seed in the middle of the insecticide band according to planting speed and band length. The precision band applicator repeats this process with each seed it detects falling through the seed tube.

In 2005, design improvements allowed band volume and band length per injection at different ground speeds to be controlled from a tractor cab mounted operator panel. Once band length and injection volume were set by the operator, these two variables

were transmitted electronically to the planter row unit via a controller area network. The row controller would read these two parameters and use them to set the "on" time of the miniature solenoid by implementing a pulse width modulation routine that controlled output as a percentage of its full delivery capacity to control injector volume and band length.

Precision Band Applicator Accuracy. Three precision band applicator accuracy trials were conducted in both 2004 and 2005 at The Ohio Agricultural Research and Development Center's (OARDC) Western Agricultural Research Station (WARS) in South Charleston, OH, to determine how often the applicator would correctly detect and inject a liquid over the target seed, establishing rates of accuracy. The pumpkin variety Magic Lantern (Harris Moran Seed Company, Modesto, CA); zucchini variety Spineless Beauty (Syngenta Seeds, Golden Valley, MN); and cucumber variety Marketmore 86 (Rupp Seeds, Wauseon, OH) were all used in both years of this trial. Because these three varieties vary considerably in size and weight, each was evaluated at planting speeds of 2.3, 3.2, and 4.6 km/h in 2004 and 3.2, 4.6, and 6.4 km/h in 2005. In both years the injection band was calibrated to release 3 ml of solution over a distance of 12.7 cm inside the furrow.

To determine the accuracy of the injected bands over the seed, each variety was "planted" on the soil surface and "banded" using only water to visually determine how accurately the band overlaid the seed. The band was delivered as a single solid stream of liquid issued from the nozzle directed at the bottom of the seed furrow. To avoid burying the seed, the planter's press wheels were removed and the planter depth adjusted to drop seeds on the soil surface in a 0.6-cm shallow furrow.

There were four replications of each treatment and each plot was 45.7 m long. All seeds regardless of type were planted at a 30–36-cm spacing. There were 100 seed and band overlap events recorded per plot. Only singulated seed were counted; any double seed drops were excluded from the data because it was impossible to tell which was the true seed and which was the extra in relation to the band. Seed dropped from the planter that landed within the injected water band on the soil surface were considered "in the band." All other outcomes such as seed landing outside the band, no seed drop, no injection band, constituted only a small fraction of the total number of events and are not reported here.

Significant modifications to the precision band applicator in 2005 made it necessary to confirm the accuracy of the injector had not been compromised. The trials, methods, data collection, and varieties were identical to those conducted in 2004 with the exception of dropping the slowest speed and adding the 6.4 km/h planting speed.

Cucurbit Seedling Bioassays. The systemic insecticide Admire 2F (imidacloprid) was used in all three pumpkin, zucchini, and cucumber bioassay studies in 2004 and 2005. Planting speed for all three bioassay studies for both years was set at 3.2 km/h. Application

rates ranged between 1.2 and 1.7 liters/ha and a base water volume of 155.3 liters/ha for all treatments, although precision banded treatments used proportionately less water volume and insecticide per hectare. Each treatment was replicated four times in a randomized complete block design. In 2004, the pumpkin plots were 45.7 m long and seeded on 10 June at a seed spacing of 40.6 cm. Both cucumber and zucchini plots were 22.8 m long, seeded on 16 June at a seed spacing of 30.5 and 38.1 cm, respectively. In 2005, the pumpkin, zucchini, and cucumber bioassay trials were all planted on 12 July. The pumpkin and zucchini plots were 39.6 m long, seeded 46 cm within-row spacing. The cucumber plots were 30.5 m long, seeded at 30.5 cm within-row spacing.

A second study on pumpkins decoupled injection volume and band length, but all treatments were derivations of the base rate of imidacloprid at 1.7 liters/ha, water carrier volume of 155.3 liters/ha at a 1.5-m row spacing. The precision banded treatments were all calibrated to inject multiples of 3 ml of insecticide solution as a single solid stream aimed in-furrow in a 12.7 cm band over the seed. Treatments varied in the total amount of active ingredient per injection, volume per injection, and the length of the application band surrounding each seed. There were four replicates of each treatment, arranged in a randomized complete block design. The pumpkin plots were 45.7 m long, seeded on 10 June at 3.2 km/h at a density of 76.2 cm.

In 2004, the "standard" treatment used in all pumpkin, zucchini, and cucumber bioassays was a continuously applied in-furrow imidacloprid rate of 1.7 liters/ha at 1.5-m row spacing. The remaining pumpkin treatments were 1) precision band applied, imidacloprid rate of 1.2 liters/ha at a row spacing of 1.5 m; 2) precision band applied imidacloprid rate of 1.7 liters/ha at a row spacing of 4.6 m; 3) continuously applied in-furrow water only check. The continuously applied treatment placed 0.108 ml (0.025 g [AI]) imidacloprid in a 40.6-cm band along each seed in the furrow. The two precision band treatments will be referred to as low and high according to their rates. Because these precision band treatments were applied in 12.7-cm bands along each seed per injection, the actual amount of imidacloprid applied to each seed was 0.0226 ml (0.0054 g [AI]) and 0.101 ml (0.024 g [AI]), respectively. These precision application rates reflect a true imidacloprid rate of 0.18 and 0.27 liters/ha at a carrier volume of 24.0 liters/ha when final seed density of 81.2 cm is taken into account.

In 2004, the remaining zucchini bioassay treatments were 1) precision band applied, imidacloprid rate of 1.21 liters/ha at 1.2-m row spacing; 2) precision band applied, imidacloprid rate of 1.7 liters/ha at 1.8-m row spacing; and 3) continuously applied in-furrow water only check. The continuously applied treatment placed 0.102 ml (0.024 g [AI]) imidacloprid in a 38.1-cm band along each seed in the furrow. The two precision band treatments are referred to as low and high according to their rates. Because these precision band treatments were applied in 12.7-cm bands along

each seed per injection, the actual amount of imidacloprid applied to each seed was 0.018 ml (0.0043 g [AI]) and 0.041 ml (0.0097 g [AI]), respectively. These precision application rates reflect a true imidacloprid rate of 0.37 and 0.55 liters/ha at a carrier volume of 24.0 liters/ha when final seed density of 38.1 cm is taken into account.

In 2004, the remaining cucumber bioassay treatments were 1) precision band applied, imidacloprid rate of 1.2 liters/ha at 0.9-m row spacing; 2) precision band applied, imidacloprid rate of 1.7 liters/ha at 1.8-m row spacing; 3) continuously applied in-furrow water only check. The continuously applied treatment placed 0.081 ml (0.019 g [AI]) imidacloprid in a 30.5-cm band along each seed in the furrow. The two precision band treatments will be referred to as low and high according to their rates. Because these precision band treatments were applied in 12.7-cm bands along each seed per injection, the actual amount of imidacloprid applied to each seed was 0.013 ml (0.0031 g [AI]) and 0.041 ml (0.0097 g [AI]). These precision application rates reflect a true imidacloprid rate of 0.46 and 0.69 liters/ha at a carrier volume of 24.0 liters/ha when the final seed density of 30.5 cm is taken into account.

After treatment of seeds in field plots in 2004, bioassays were conducted on pumpkin, zucchini, and cucumber seedlings at the fully expanded cotyledon, first, and second leaf to test for the presence of imidacloprid. In addition, zucchini seedlings were also bioassayed at the fifth leaf. There were five leaf samples taken from each replicate plot for a total of 20 samples per treatment or 80 samples per bioassay. Seedling leaf stages were determined when 50% or more of the seedlings in the trial reached that stage. As the seedlings grew from cotyledon to first leaf to second leaf, and so on, five randomly chosen plants had their most recently developed leaf clipped from the plant at the base of the petiole using scissors and placed in a clear 0.24 liters clear plastic container (Solo, Highland Park, IL). Immediately after placing the clipped leaf inside the container, the container was placed inside a cardboard box to prevent wilting from the heat and sun. Within 30 min, all 80 freshly clipped leaf samples for one bioassay were collected and transported inside a nearby building to be kept cool and maintain tissue turgor. The partially dissected plant in the field was then destroyed to prevent further sampling at a later date.

To complete the bioassays, striped cucumber beetle adults, *Acalymma vittatum* (F.), were collected at WARS from three isolated areas ≈ 9.1 by 30.5 m seeded the first week of May with a mixture of pumpkin and squash to serve as cucumber beetle reservoirs. No pesticides were used in establishing these plantings, nor were any pesticides used in these areas throughout the duration of the bioassay experiments. Based on the dates of the bioassays, the majority of beetles collected were from the over wintering generation.

On the days scheduled for a bioassay trial, striped cucumber beetles were manually aspirated using model 1135A (Bioquip, Rancho Dominguez, CA) off

foliage and out of flowers into 9 dram plastic vials from the early planted cucurbit reservoir area between 7:00 a.m. and 10:30 a.m.. Approximately ten beetles were placed within each 9 dram plastic vial. Fifteen to 30 min were required to collect between 80 and 100 beetles to supply one bioassay experiment. Collected beetles were immediately placed in refrigeration at 7°C to slow their physical activity. Most beetles were used in a bioassay experiment within three hours of being field collected.

During bioassay assembly, only one vial containing striped cucumber beetles was removed from the refrigerator at a time to maintain their lethargy. One striped cucumber beetle adult was placed into a clear plastic dish that contained a recently cut seedling leaf, then sealed with the lid. The containers were stored at room temperature, $\approx 24^\circ\text{C}$ for the duration of the bioassay.

The pumpkin bioassay trial treatments in 2005 were the same as 2004 except the "standard" treatment was replaced with 1) continuously applied in-furrow, imidacloprid rate of 1.2 liters/ha at a row spacing of 1.5 m (low); and 2) continuously applied in-furrow, imidacloprid rate of 1.7 liters/ha at row spacing 4.6 m (high) which exposed each seed to 0.0813 ml (0.019 g [AI]) and 0.363 ml (0.086 g [AI]), respectively.

The zucchini bioassay trial treatments in 2005 were the same as 2004 except the "standard" treatment was replaced with 1) continuously applied in-furrow, imidacloprid rate of 1.2 liters/ha at a row spacing of 1.2 m (low); and 2) continuously applied in-furrow, imidacloprid rate of 1.7 liters/ha at a row spacing of 1.8 m (high) which exposed each seed to 0.065 ml (0.015 g [AI]) and 0.146 ml (0.034 g [AI]), respectively.

The cucumber bioassay trial treatments in 2005 were the same as 2004 except the "standard" treatment was replaced with 1) continuously applied in-furrow, imidacloprid rate of 1.2 liters/ha at a row spacing of 0.9 m (low); and 2) continuously applied in-furrow, imidacloprid rate of 1.7 liters/ha at a row spacing of 1.8 m (high), which exposed each seed to 0.032 ml (0.0077 g [AI]) and 0.097 ml (0.023 g [AI]), respectively.

Treatments for the band length and volume study in 2005 were as follows: 1) 3 ml of insecticide solution (0.034 ml of imidacloprid (0.00811 g [AI]) applied in a 12.7-cm band (short band, low volume); 2) 9 ml of insecticide solution (0.102 ml of imidacloprid (0.0241 g [AI]) applied in a 12.7-cm band (short band, high volume); 3) 18 ml of insecticide solution (0.204 ml of imidacloprid (0.0487 g [AI]) applied in a 25.4-cm band (medium band, high volume); 4) continuous in-furrow application at the rate of 18 ml of insecticide solution (0.204 ml imidacloprid (0.0487 g [AI]) per 76-cm band (long band, high volume); and 5. a water only check applied continuously in-furrow at 18 ml per 76-cm band.

In 2005, bioassays conducted with continuous and precision banded treated seeds followed the same protocol as 2004 with the following changes. Pumpkin, zucchini, and cucumber seedlings were bioassayed at cotyledon, first, second, and third leaf. In addition,

Table 1. Mean percentage of accuracy of the precision band applicator and seed drop between three cucurbit seed types at three different speeds in 2004 and 2005

Planting speed	Seeds in injection ^a band (%)					
	2004			2005		
	Pumpkin	Zucchini	Cucumber	Pumpkin	Zucchini	Cucumber
2.3 km/h	97.0 ± 1.1a	96.0 ± 1.7a	91.8 ± 1.8a			
3.2 km/h	94.0 ± 1.1a	97.3 ± 1.1a	93.8 ± 0.5a	97.3 ± 0.9b	96.0 ± 1.1a	95.0 ± 0.7a
4.6 km/h	93.8 ± 1.0a	89.5 ± 1.0b	92.5 ± 0.9a	94.0 ± 1.1ab	97.5 ± 1.2a	93.8 ± 0.9a
6.4 km/h				90.0 ± 1.8a	95.0 ± 0.6a	92.5 ± 0.3a
Mean	94.9 ± 0.7	94.3 ± 1.2	92.7 ± 0.7	93.8 ± 1.1	96.2 ± 0.6a	93.8 ± 0.5a

^a Each injection contains 3 ml of water per 12.7-cm band.

zucchini and cucumber seedlings were bioassayed at the fourth leaf. There were 10 leaf tissue sub samples taken from each replicate plot for a total of 40 samples per treatment in an effort to increase the sensitivity of the bioassays.

In 2005, the band length and volume study, bioassay trials were conducted on pumpkin at the cotyledon, first, second, third, and fourth leaf using the same protocol established earlier in 2005, including 10 sub-samples per replicate plot, totaling 40 samples per treatment.

Beetle mortality for all studies in 2004 and 2005 were recorded at 24, 48, and 72 h after initial confinement but only the 72-h data are reported here. In each bioassay dish, dead striped cucumber beetles were typically discernible from live ones by their lack of movement. Beetle leaf feeding was not quantified during the bioassay, however all beetles did feed and were subsequently exposed to any toxins present within the leaf. After 72 h, some intoxicated beetles could not be categorized as absolutely dead because they could slowly move an antennae or leg, but because they could not walk, feed, or mate, they were categorized as functionally dead for the purposes of this study. Analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test was used to separate the treatment mean mortalities after 72 h (XL Stat 7.5; Addinsoft, New York).

Results and Discussion

Precision Band Applicator Accuracy. Tests conducted at the agricultural engineering research laboratory in Columbus, OH, confirmed that seed dropped through the planter's seed tube would initiate a cascade of electrical and mechanical events resulting in an injection of liquid from the miniature solenoid controlled banding nozzle. It was paramount to the success of the project that this injection band be synchronized with the seed drop as it entered the furrow under normal planting conditions.

In 2004, only the zucchini trial showed significant differences for decreased accuracy at the 4.6 km/h planting speed ($F = 18.74$; $df = 2, 11$; $P = 0.003$) (Table 1). The initial design of the applicator did not allow for modulation of the volume within a specified band length at a specific speed, meaning the treatment band lengthened from 12.7 to 13–15 cm at 3.2 km/h, and

15–18 cm at the highest speed of 4.6 km/h, possibly overestimating accuracy at higher speeds. As the actual seed size decreased from pumpkin to cucumber, so did the mean accuracy of the precision bander to properly target the seed.

In 2005, there were significant differences between banding accuracies at different speeds ($F = 7.56$; $df = 2, 11$; $P = 0.012$) for pumpkin (Table 1). Unlike in 2004, the precision bander accuracy trials in 2005 did not strongly link seed size and accuracy. The new operator interface, control module, and constant band length may have contributed to the slightly different accuracy means.

Differences in banding accuracies may be attributed to a lack of detection of smaller seeds by the optical sensor in the seed tube. Other possible factors may include seed momentum at increased planting speeds caused the seed to bounce or skip from its original landing location, or the injection stream itself flushed the seed out of the shallow furrow.

Cucurbit Seedling Bioassays. Imidacloprid applied in-furrow with the seed will be absorbed by the seedling roots and translocated systemically, effectively administering lethal or sub-lethal insecticide doses to beetles feeding on the leaf tissue. Evidence of imidacloprid in the seedling can be inferred from bioassays in which striped cucumber beetles are fed tissue grown from seeds exposed to in-furrow applications of imidacloprid. Striped cucumber beetles fed leaf tissue containing imidacloprid should have a lower survival compared with beetles fed untreated leaves.

In the three pumpkin bioassay trials of 2004 (Table 2), both the standard and high treatments had significantly higher beetle mortality than the check at the cotyledon stage ($F = 10.45$; $df = 3, 15$; $P = 0.003$). Beetle mortality was significantly higher in all treatments compared with the check at the first leaf ($F = 32.44$; $df = 3, 15$; $P < 0.0001$).

In the four zucchini bioassay trials of 2004 (Table 3), there were significant differences among treatments for beetle mortality at the cotyledon, first leaf, and second leaf compared with the check ($F = 69.66$; $df = 3, 15$; $P < 0.0001$; $F = 31.33$; $df = 3, 15$; $P < 0.0001$; and $F = 12.89$; $df = 3, 15$; $P = 0.001$). Only at the first leaf did the standard and high treatments have significantly higher mortality than the low treatment.

In the three cucumber bioassay trials of 2004 (Table 4), all cotyledon, first leaf, and second leaf treatments

Table 2. Mean bioassay mortality of striped cucumber beetles on pumpkin after 72-h exposure to seedling stages grown from seeds treated with imidacloprid solution in-furrow by precision banding or continuous application in 2004

Treatment	Rate ^a	Rate ^b	% mortality ± SE		
			Cotyledon	First leaf	Second leaf
Check			0.0 ± 0.0a	0.0 ± 0.0a	5.0 ± 5.0a
Low ^c	1.2	0.18	35.0 ± 9.6ab	40.0 ± 11.5b	5.0 ± 5.0a
Standard ^d	1.7	1.7	45.0 ± 15.0b	63.2 ± 13.1bc	35.0 ± 9.6a
High ^c	1.7	0.27	70.0 ± 5.8b	90.0 ± 5.8c	35.0 ± 17.1a

Treatment means followed by same letter are not significantly different ($P > 0.05$).

^a Initial insecticide rate (liters/ha) based on product label.
^b Actual insecticide rate (liters/ha) applied considering precision application.

^c Insecticide applied in a 3-ml 12.7-cm precision band over the seed at the bottom of the furrow.

^d Insecticide applied in a continuous stream over the seed at the bottom of the furrow.

had significantly higher beetle mortality than the check ($F = 17.55$; $df = 3, 15$; $P = 0.0004$; $F = 19.87$; $df = 3, 15$; $P = 0.0002$; $F = 13.68$; $df = 3, 15$; $P = 0.001$).

In each bioassay experiment there were treatments, excluding the check, where up to 8% of beetles that seemed dead at one evaluation revived at a later evaluation. This revival or knockdown phenomenon has been noted in adult Japanese beetle, *Popillia japonica* Newman, studies where imidacloprid was used as the intoxicant (Wise et al. 2007). Similar imidacloprid intoxication effects, including loss of coordination, leg movements, and paralysis were seen by Kunkel et al. (2001) and Nauen (1995) working with carabid beetles and aphids, respectively. The intoxicated striped cucumber beetles were immobile and could only slowly move their legs or antennae.

The narrow row spacing and low labeled insecticide rate and a wide row spacing at the high labeled insecticide rate were selected for each group of cucurbit treatments to effectively bracket the lowest to highest in-row concentration of active ingredient per linear meter. Beetle mortality at the low and high rates should provide estimates of corresponding mortality at any row spacing and insecticide rate combination within those parameters. In 2004, the in-furrow ppm concentration of imidacloprid ranged between 1,076 and 7,816 for all treatments (Table 5). The formula to calculate ppm is the (total volume of imidacloprid

Table 4. Mean bioassay mortality of striped cucumber beetles on cucumber after 72-h exposure to seedling stages grown from seeds treated with imidacloprid solution in-furrow by precision banding or continuous application in 2004

Treatment	Rate ^a	Rate ^b	% mortality ± SE		
			Cotyledon	First leaf	Second leaf
Check			15.0 ± 15.0a	35.0 ± 9.6a	10.0 ± 5.8a
Low ^c	1.2	0.46	85.0 ± 9.6b	70.0 ± 5.8b	65.0 ± 12.6b
Standard ^d	1.7	1.7	100.0 ± 0.0b	100.0 ± 0.0b	90.0 ± 5.8b
High ^c	1.7	0.71	95.0 ± 5.0b	95.0 ± 5.0b	75.0 ± 9.6b

Treatment means followed by same letter are not significantly different ($P > 0.05$).

^a Initial insecticide rate (liters/ha) based on product label.
^b Actual insecticide rate (liters/ha) applied considering precision application.

^c Insecticide applied in a 3-ml 12.7-cm precision band over the seed at the bottom of the furrow.

^d Insecticide applied in a continuous stream over the seed at the bottom of the furrow.

[ml] \times 0.239 [converts 1 ml of product to g [AI]/ml] / ((total volume water ([ml]) + (total volume of imidacloprid [ml] \times 1.12 [specific gravity of imidacloprid])) \times 1,000,000.

In 2004, the potential environmental impact and economic savings in pumpkin, zucchini, and cucumber by using the high and low precision banded application rates reduced imidacloprid on a per hectare basis by 84.5, 66.6, and 58.3%, respectively, compared with continuous application of the product at the same label rate (Table 5). The economic savings of precision banding reduced insecticide costs of pumpkin, zucchini, and cucumber crops up to \$215, \$169, and \$148/ha, respectively, compared with the current maximum labeled rate of Admire 2F at 1.75 liters/ha applied continuously in-furrow (Table 5). Using precision banding also reduces the amount of water needed as a carrier, enabling more acres to be treated without refilling the carrier tank.

In 2005, there were four pumpkin leaf bioassays. All treatments had significantly higher mortality than the check at the cotyledon ($F = 249.36$; $df = 4, 19$; $P < 0.0001$) and first leaf ($F = 23.97$; $df = 4, 19$; $P < 0.0001$) (Table 6). At the second and third leaf, both high rate treatments had significantly higher mortality than the check ($F = 10.74$; $df = 4, 19$; $P = 0.001$; and $F = 6.59$; $df = 4, 19$; $P = 0.005$).

Table 3. Mean bioassay mortality of striped cucumber beetles on zucchini after 72-h exposure to seedling stages grown from seeds treated with imidacloprid solution in-furrow by precision banding or continuous application in 2004

Treatment	Rate ^a	Rate ^b	% mortality ± SE			
			Cotyledon	First leaf	Second leaf	Fifth leaf
Check			10.5 ± 5.8a	0.0 ± 0.0a	5.0 ± 5.0a	5.0 ± 5.0a
Low ^c	1.2	0.37	95.0 ± 5.0b	45.0 ± 5.0b	50.0 ± 12.9b	30.0 ± 19.1a
Standard ^d	1.7	1.70	95.0 ± 5.0b	78.9 ± 8.3c	80.0 ± 11.5b	35.0 ± 12.6a
High ^c	1.7	0.57	90.0 ± 5.8b	85.0 ± 9.6c	47.4 ± 4.8b	40.0 ± 14.1a

Treatment means followed by same letter are not significantly different ($P > 0.05$).

^a Initial insecticide rate (liters/ha) based on product label.
^b Actual insecticide rate (liters/ha) applied considering precision application.
^c Insecticide applied in a 3-ml 12.7-cm precision band over the seed at the bottom of the furrow.
^d Insecticide applied in a continuous stream over the seed at the bottom of the furrow.

Table 5. Comparing imidacloprid usage and cost per ha between precision band applied and continuous in-furrow applications in 2004

Crop	Rate ^a	Rate ^b	ppm solution	Reduction/ha (% [AI])	Cost ^c /ha	Savings ^c /ha
Pumpkin ^d	1.2	0.18	1,788	84.5	\$26.23	\$143.70
Pumpkin ^e	1.7	1.7	2,671	0.0	\$254.90	\$0
Pumpkin ^d	1.7	0.27	7,816	84.5	\$39.51	\$215.39
Zucchini ^d	1.2	0.37	1,433	66.6	\$56.75	\$113.17
Zucchini ^e	1.7	1.7	2,671	0.0	\$254.90	\$0
Zucchini ^d	1.7	0.57	3,197	66.6	\$84.96	\$169.94
Cucumber ^d	1.2	0.46	1,076	58.3	\$70.81	\$ 99.12
Cucumber ^e	1.7	1.7	2,671	0.0	\$254.90	\$0
Cucumber ^d	1.7	0.71	3,197	58.3	\$106.21	\$148.69

^a Initial insecticide rate (liters/ha) based on product label.

^b Actual insecticide rate (liters/ha) applied considering application method.

^c Imidacloprid calculated at \$4.30/29.5 ml.

^d Precision applied at 3 ml in a 12.7-cm band.

^e Continuous in-furrow application.

In 2005, there were five zucchini leaf bioassays. All treatments had significantly higher mortality than the check at the cotyledon ($F = 152.31$; $df = 4, 19$; $P < 0.0001$) and first leaf ($F = 21.48$; $df = 4, 19$; $P < 0.0001$) (Table 7). At the second leaf, both high rate treatments had significantly higher mortality than the check ($F = 7.22$; $df = 4, 19$; $P = 0.003$). There was no significant difference between the treatments at the third leaf ($F = 2.20$; $df = 4, 19$; $P = 0.130$) or fourth leaf ($F = 2.15$; $df = 4, 19$; $P = 0.136$).

In 2005, there were five cucumber leaf bioassays. All treatments at the cotyledon ($F = 67.19$; $df = 4, 19$; $P < 0.0001$) and first leaf ($F = 24.52$; $df = 4, 19$; $P < 0.0001$) had beetle mortality significantly higher than the check (Table 8). At the second leaf, only the high precision band treatment had significantly higher mortality than the check ($F = 5.53$; $df = 4, 19$; $P = 0.009$). At the third leaf both high rate treatments had significantly higher mortality than the check ($F = 7.27$; $df = 4, 19$; $P = 0.004$). There were no significant differences between any treatments at the fourth leaf ($F = 0.76$; $df = 4, 19$; $P = 0.57$).

In previous pumpkin, zucchini, and cucumber bioassays conducted in 2005, within each pair of low or high rate treatments, there was no significant difference in beetle mortality between the application techniques, i.e., precision banded or continuous flow. In each bioassay experiment in 2005, up to 4.5% of beetles in treatments excluding the check demonstrated signs

of being “knocked down” at one evaluation only to be deemed alive at a later evaluation, similar to the 2004 bioassay observations.

In 2005, the potential environmental impact and economic savings in pumpkin, zucchini, and cucumber using precision banded application rates reduced imidacloprid on a per hectare basis by 83.3, 72.2, and 58.3%, respectively, compared with continuous application at the same label rate (Table 9). The economic benefit of using precision banding can reduce insecticide costs on pumpkin, zucchini, and cucumber crops up to \$212, \$184, and \$148/ha, respectively, compared with the current maximum labeled rate of Admire 2F at 1.75 liters/ha applied continuously in-furrow (Table 9).

The ppm concentration of imidacloprid solution ranged from 1,076 to 7,816 among the treatments based on row width and the initial insecticide rate for each pumpkin, zucchini, and cucumber bioassay trial (Table 9). Between the two application methods, the total amount of active ingredient available to each seed was greater in the continuous in-furrow treatments due to the length of the furrow being treated, 76.2 cm compared with only 12.7 cm of the furrow in the precision banded treatments. The extra imidacloprid would be most accessible later in the season with appropriate moisture but not likely to affect efficacy at early seedling leaf stages.

Table 6. Mean bioassay mortality of striped cucumber beetles on pumpkin after 72-h exposure to seedling stages grown from seeds treated with imidacloprid solution in-furrow by precision banding or continuous application in 2005

Treatment	Rate ^a	Rate ^b	% mortality \pm SE			
			Cotyledon	First leaf	Second leaf	Third leaf
Check			5.0 \pm 2.9a	5.6 \pm 3.3a	0.0 \pm 0.0a	0.0 \pm 0.0a
Low band ^c	1.2	0.18	95.0 \pm 2.9b	74.4 \pm 13.8b	23.1 \pm 6.2ab	7.5 \pm 4.8a
Low con't ^d	1.2	1.2	100.0 \pm 0.0b	81.1 \pm 6.9b	25.8 \pm 5.8ab	0.0 \pm 0.0a
High band ^c	1.7	0.27	97.5 \pm 2.5b	87.5 \pm 7.2b	43.9 \pm 3.9bc	30.0 \pm 4.1b
High con't ^d	1.7	1.7	97.5 \pm 2.5b	91.9 \pm 5.3b	55.2 \pm 13.3c	30.6 \pm 10.6b

Treatment means followed by same letter are not significantly different ($P > 0.05$).

^a Initial insecticide rate (liters/ha) based on product label.

^b Actual insecticide rate (liters/ha) applied considering precision application.

^c Insecticide applied in a 3-ml 12.7-cm precision band over the seed at the bottom of the furrow.

^d Insecticide applied in a continuous stream over the seed at the bottom of the furrow.

Table 7. Mean bioassay mortality of striped cucumber beetles on zucchini after 72-h exposure to seedling stages grown from seeds treated with imidacloprid solution in-furrow by precision banding or continuous application in 2005

Treatment	Rate ^a	Rate ^b	% mortality \pm SE				
			Cotyledon	First leaf	Second leaf	Third leaf	Fourth leaf
Check			23.9 \pm 3.2a	7.5 \pm 4.8a	0.0 \pm 0.0a	0.0 \pm 0.0a	28.3 \pm 6.5a
Low band ^c	1.2	0.37	97.5 \pm 2.5b	80.0 \pm 4.1b	24.4 \pm 9.6ab	20.0 \pm 8.2a	35.0 \pm 8.7a
Low con't ^d	1.2	1.2	90.0 \pm 5.8b	80.0 \pm 7.1b	20.0 \pm 4.1ab	10.8 \pm 7.9a	47.5 \pm 8.5a
High band ^c	1.7	0.57	97.5 \pm 2.5b	90.0 \pm 7.1b	45.0 \pm 5.0b	20.6 \pm 4.1a	55.0 \pm 9.6a
High con't ^d	1.7	1.7	100.0 \pm 0.0b	90.0 \pm 10.0b	33.9 \pm 11.2b	10.3 \pm 4.1a	61.4 \pm 13.3a

Treatment means followed by same letter are not significantly different ($P > 0.05$).

^a Initial insecticide rate (liters/ha) based on product label.

^b Actual insecticide rate (liters/ha) applied considering precision application.

^c Insecticide applied in a 3-ml 12.7-cm precision band over the seed at the bottom of the furrow.

^d Insecticide applied in a continuous stream over the seed at the bottom of the furrow.

Considering the results of the pumpkin, zucchini, and cucumber bioassays, in general, treatments with a higher ppm dose of imidacloprid had greater beetle mortality in bioassays than treatments at lower rates, particularly at later leaf stages. There were no significant differences in the percent mortality by application type at the same rate in all 12 bioassay trials in 2005, meaning the efficacy of precision banded and continuously applied treatments were equivalent at the same rate.

The redesigned precision band applicator allowed us the flexibility to vary the volume per injection and band length independently. By maintaining a constant concentration, it was possible to see how each component affected beetle control in pumpkin leaf bioassays. In this trial the rate was based on 1.7 liters/ha of imidacloprid applied at 155.3 liters/ha. Only the distribution within the seed furrow, total amount of active ingredient and volume applied relative to each seed differed between treatments. The imidacloprid solution concentration remained at 2,605 ppm for all treatments.

All bioassay treatments at the cotyledon ($F = 83.50$; $df = 4, 19$; $P < 0.0001$), first leaf ($F = 10.13$; $df = 4, 19$; $P = 0.0003$), third leaf ($F = 4.70$; $df = 4, 19$; $P = 0.0095$), and fourth leaf ($F = 8.61$; $df = 4, 19$; $P = 0.0007$) had significantly higher beetle mortality compared with the check (Table 10). At the second leaf, all treatments except the long band high volume had significantly higher beetle mortality than the check ($F = 6.89$; $df = 4, 19$; $P = 0.002$). In each bioassay up to 5.3%

of the beetles in treatments except for the check seemed "knocked down" at one evaluation but revived at a later evaluation, an occurrence noted in earlier bioassays.

The long band high volume treatment was actually applied as a continuous in-furrow stream equivalent to consecutive 76-cm bands, whereas the medium band high volume was precision applied in 25.4-cm intervals; both contained 18 ml per injection. In four of the five leaf bioassay trials there was no significant difference in beetle mortality between these treatments except at the second leaf where there was significantly less mortality in the long banded treatment. With only two treatments testing the effect of band length at constant volume on mortality, the results indicate little evidence for a significant effect. Between these two treatments, the medium band treatment had higher beetle mortality in 80% of the leaf bioassays.

The short band treatments compared the affects of increased injection volume on beetle mortality while keeping the band length constant at 12.7 cm. There were no significant differences in any of the bioassays or apparent trends in beetle mortality between these two treatments.

Despite having a six-fold difference in total carrier volume and insecticide, the long band high volume is clearly not superior to the short band low volume treatment. In actuality, both treatments have the same active ingredient and carrier volume per linear centimeters. This may suggest that seedlings are only able to access imidacloprid within a certain zone from the

Table 8. Mean bioassay mortality of striped cucumber beetles on cucumber after 72-h exposure to seedling stages grown from seeds treated with imidacloprid solution in-furrow by precision banding or continuous application in 2005

Treatment	Rate ^a	Rate ^b	% mortality \pm SE				
			Cotyledon	First leaf	Second leaf	Third leaf	Fourth leaf
Check			15.0 \pm 6.5a	2.7 \pm 2.5a	2.6 \pm 2.8a	0.0 \pm 0.0a	7.9 \pm 4.8a
Low band ^c	1.2	0.46	87.5 \pm 12.5b	45.9 \pm 6.1b	12.5 \pm 4.8ab	20.0 \pm 1.3a	7.7 \pm 2.6a
Low con't ^d	1.2	1.2	100.0 \pm 0.0b	60.5 \pm 6.2bc	7.5 \pm 4.8a	28.9 \pm 4.1ab	5.1 \pm 5.6a
High band ^c	1.7	0.71	95.0 \pm 5.0b	60.0 \pm 4.1bc	30.0 \pm 7.1b	50.0 \pm 10.8b	0.0 \pm 0.0a
High con't ^d	1.7	1.7	97.5 \pm 2.5b	81.1 \pm 7.5c	23.7 \pm 3.2ab	52.5 \pm 10.3b	5.1 \pm 2.9a

Treatment means followed by same letter are not significantly different ($P > 0.05$).

^a Initial insecticide rate (liters/ha) based on product label.

^b Actual insecticide rate (liters/ha) applied considering precision application.

^c Insecticide applied in a 3-ml 12.7-cm precision band over the seed at the bottom of the furrow.

^d Insecticide applied in a continuous stream over the seed at the bottom of the furrow.

Table 9. Economics and insecticide reduction of precision banded treatments compared with continuous flow application of Admire 2F in 2005

Crop	Rate ^a (l/ha)	Rate ^b (l/ha)	ppm solution	Reduction/ha (% [AI])	Cost/ha ^c (\$)	Savings/ha ^c (\$)
Pumpkin ^d	1.2	0.18	1,788	83.3	28.33	141.61
Pumpkin ^e	1.2	1.2	1,788	0	169.94	\$0
Pumpkin ^d	1.7	0.27	7,816	83.3	42.48	212.42
Pumpkin ^e	1.7	1.7	7,816	0	254.90	\$0
Zucchini ^d	1.2	0.37	1,433	72.2	47.20	122.74
Zucchini ^e	1.2	1.2	1,433	0	169.94	\$0
Zucchini ^d	1.7	0.57	3,197	72.2	70.81	184.09
Zucchini ^e	1.7	1.7	3,197	0	254.90	0
Cucumber ^d	1.2	0.46	1,076	58.3	70.81	99.13
Cucumber ^e	1.2	1.2	1,076	0	169.94	0
Cucumber ^d	1.7	0.71	3,197	58.3	106.21	148.69
Cucumber ^e	1.7	1.7	3,197	0	254.90	0

^a Initial insecticide rate based on product label.
^b Actual insecticide rate applied considering precision application.
^c Admire 2F product priced at \$4.30/29.5 ml.
^d Insecticide applied in a 3-ml 12.7 cm precision band over the seed at the bottom of the furrow.
^e Insecticide applied in a continuous stream over the seed at the bottom of the furrow.

seedling as determined the developing root mass. We have observed the trend of higher carrier volumes and increased active ingredient per band in close proximity of the seed lengthening the control window and increasing beetle mortality. The precision band applicator allowed us the flexibility to manage the application to maximize pesticide reduction or extend the window of beetle control.

We have confidence that the precision band applicator used in conjunction with a systemic insecticide to treat direct seeded cucurbits in-furrow can control striped cucumber beetles under field conditions, even though it was only evaluated using lab bioassays. The precision bander accurately treated >90% of the cucurbit seeds in our trials at several planting speeds. This does allow for up to 10% of seeds and seedlings to go untreated in the field which under moderate to heavy beetle feeding pressure in the spring or early summer, can cause these unprotected plants to die from excessive feeding damage or require a foliar spray to control the beetles. Bacterial wilt transmission is also an important factor to consider monitoring

more closely in the field, especially given the susceptibility to this disease varies dramatically among cucurbit species. The uptake of imidacloprid into the plant also can be affected by the amount of precipitation or irrigation received, with low amounts of either leading to roots that grow beyond the zone of the applied chemical, potentially limiting the overall amount of material absorbed into the plant. No irrigation was supplied to the field plots in 2004 or 2005. This may explain why most bioassay mortalities dropped off substantially after the first leaf.

Using the precision bander, we have managed to substantially reduce imidacloprid in the environment and decrease the cost per hectare compared with conventional application while maintaining equivalent striped cucumber beetle mortality measured by leaf bioassays conducted in the lab. How beetle mortality would respond under strictly field conditions is worth further investigation, especially as we move toward systemic insecticide seed treatment technologies.

Table 10. Mean bioassay mortality of striped cucumber beetles after 72 h exposure to imidacloprid^a-treated pumpkin leaf tissue

Treatment	Rate ^a	Rate ^b	Band length (cm)	Volume/band (ml)	% mortality ± SE				
					Cotyledon	First leaf	Second leaf	Third leaf	Fourth leaf
Check			76.2	18	13.8 ± 3.8b	0.0 ± 0.0b	2.5 ± 2.5c	2.5 ± 2.5c	0.0 ± 0.0c
Short band low volume ^c	1.7	0.29	12.7	3	100.0 ± 0.0a	61.7 ± 13.4a	35.3 ± 5.5ab	60.0 ± 9.1ab	32.5 ± 4.8b
Short band medium volume ^c	1.7	0.87	12.7	9	97.5 ± 2.5a	74.4 ± 6.3a	34.4 ± 5.3b	47.5 ± 14.9b	47.5 ± 2.5ab
Medium band high volume ^c	1.7	1.7	25.4	18	92.5 ± 4.8a	81.9 ± 6.4a	52.2 ± 6.1a	89.4 ± 7.1a	56.7 ± 8.8a
Long band high volume ^d	1.7	1.7	76.2	18	100.0 ± 0.0a	65.8 ± 9.3a	18.1 ± 8.6bc	56.3 ± 15.9ab	43.2 ± 8.5ab

Treatment means followed by same letter are not significantly different ($P > 0.05$).
^a Imidacloprid concentration for all treatments is 2,605 ppm.
^b Initial insecticide rate (liters/ha) based on product label.
^c Actual insecticide rate (liters/ha) applied considering precision application.
^d Insecticide applied in a 3-ml 12.7-cm precision band over the seed at the bottom of the furrow.
^e Insecticide applied in a continuous stream over the seed at the bottom of the furrow.

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